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(54) **Method and apparatus for detecting biological activities in a specimen.**

(57) A method, a sensor and apparatus for detecting biological activities in a specimen, for example in a blood sample, are provided in which a sealable container is sealed with a culture medium therein into which the sample is introduced, metabolic processes are enhanced in the presence of microorganisms in the sample and changes taking place in the concentrations of the substances such to such processes are detected and monitored with an excitation and detection assembly assigned to concentration sensors, herein the form of optodes which are optically coupled to the excitation and detection assembly and to thereby to an evaluation unit for determining concentration changes of the substances over time as indications of the presence of microorganisms.

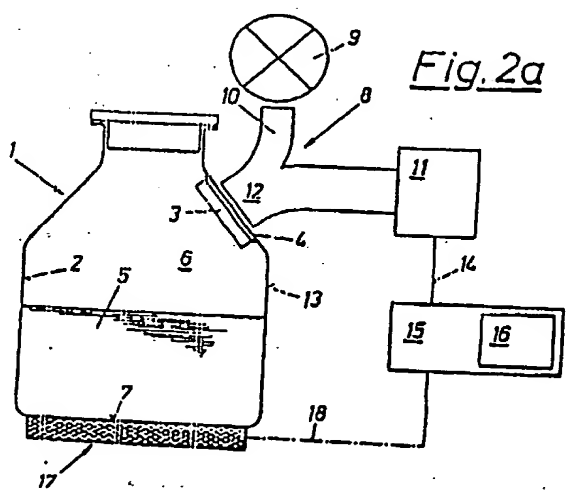

Fig.2a

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The present invention relates to a m thod and to an apparatus for detecting biological activiti s in a specimen where the specimen and a culture medium, are introduced into a sealable container and are exposed to conditions enabling metabolic processes to take place in the presence of microorganisms in the sample, the concentration of the initial substances being lowered and that of metabolic products being raised.

In many applications it is necessary to determine quickly whether a specimen is contaminated by microorganisms, such as bacteria, in particular in medical applications, in the pharmaceutical industry, food industry, or in environmental protection activities. The term "specimen" has a most comprehensive meaning here, including substances such as solid and liquid biological material (e.g. blood), food samples, such as frozen foods and preserves of canned foods, packaging material, clinical instruments and laboratory equipment, or samples taken from their surfaces, medical apparatus, first-aid and dressing material, soil and water samples, particularly samples of drinking water.

For a long time purely manual methods have been used in which the specimen to be assessed is placed in a culture bottle containing a liquid culture medium, and the growth of the culture is inspected only visually at given time intervals, and the type of presence of a microorganism is inferred from this observation by subculturing the liquid culture medium to a solid culture medium.

In addition, some technical procedures and devices are known, with which the biological activities in a sample are caused by microorganisms may be determined, and where the CO_2 produced by the metabolism of the microorganism, or rather, the change in CO_2 content, is employed as a measurement for determining the bological activity.

It is a known procedure, for example, to bottle the sample to be assessed together with a radioactiv ly-labeled liquid culture medium and to test the atmosphere over the culture medium for radioactive gases, following which the presence of microorganisms in the sample may be determined.

Measuring systems of this type are described in U.S. 3,076,679 and 3,935,073, for example, fully incorporated herein by this reference. Although such systems are quick and reliable, they have certain disadvantages, i.e. radioactive substances must be handled and samples must be repeatedly taken from the gas space above the culture medium for frequent monitoring. When the samples are removed from the gas space, the remaining samples to be monitored may easily be contaminated by the sample-taking element and measuring errors may occur.

In the European application 158 497, a system is disclosed in which the biological activity of the specimen is determined by means of infrared absorption. In this method, a specimen is introduced into a sealable vessel containing a liquid culture medium, and is tested for the presence of microorganisms. The vessel is subjected to specific conditions, i.e. certain temperatures are maintained over given periods of time, thus enhancing the metabolism of the microorganisms, during which process CO_2 is produced in the gas space above the culture medium by conversion of the carbon source. A sample is taken from the gas space and introduced into a measuring cell, and the CO_2 content is measured by infrared absorption. Again, the subsequent samples may be contaminated, and another drawback is that infrared absorption is a less-sensitive means of measuring than radioactive labeling.

In order to avoid the problem of cross-contamination, the European application 0 104 463 proposes a method and a device which are also based on the detection of CO_2 (produced by metabolic processes) by means of infrared absorption. In this method, no sample is taken, but infrared radiation is directly transmitted through the wall of the vessel into the gas space above the culture medium, and its absorption is determined. Due to this non-invasive measuring method, cross-contaminations are largely eliminated; the disadvantage of this method, however, is its lack of sensitivity compared to radiometric methods, as well as the fact that the measurement is distorted by other gas components absorbing radiation in the same frequency band as CO_2. A suitable example is the absorption bands of water vapor. The sample vessels employed must be permeable within a relatively narrow frequency range, which will only permit the use of specific materials for these vessels. An additional advantage is that the generation and filtering of the required infrared radiation is comparatively complex and expensive.

The European application 0 333 253 describes a device and apparatus for monitoring changes in pH and CO_2 in a bacterial culture utilizing optical absorption measurements of a pH indicator in a matrix. Although it is not as sensitive as the radiometric method, it does offer the advantage of being noninvasive and can be continuously monitored. The primary disadvantage is that since color changes are being measured, different optical systems must then be used when the indicator medium is changed, thereby limiting the apparatus to one or two sensors.

It is therefore an object of the present invention to provide a method and an apparatus for detecting biological activities in a specimen, which have at least the same sensitivity as radiometric methods, and which offer the user a versatile, simple and inexpensive technique, providing him with information on the

presence of microorganisms, while eliminating the danger of cross-contamination.

According to the present invention, the above object is achieved by continuously measuring the concentration of at least one (produced or consumed) substance subject to conversion by metabolic processes, generating a test signal by way of optodes in direct contact with the substances to be assessed; and monitoring the changes over time of the test signal or signals to serve as an indicator for the presenc of microorganisms. The optical sensors or optodes which rely on the principle of fluorescence attenuation or enhancement, which may be inexpensively mass produced and which are in direct contact with th substance to be assessed and attached to a transparent surface of the container (internal filtered effect), permit continuous monitoring in closed systems, where new optodes are used for each sample, such that cross-contaminations are eliminated in the most simple variation, for example, a single sensor may suffice to determine the presence of microorganisms by way of the changes in substance concentrations relative to the initial concentrations.

With respect to the aforementioned disadvantage of measuring color changes and the requirement for different optical systems when an indicator medium is changed, another object of the present invention is to provide a sensor which allows the monitoring of many optical changes while utilizing the same optical system.

According to the present invention, the immediately-foregoing object is achieved by utilizing an inert fluorophore in the optode and measuring the modulation of the fluorescent output caused by optical changes in the indicator medium. It also provides for very sensitive continuously monitoring a noninvasive system.

The present invention provides that concentrations be measured of at least one substance from the group of CO_2, O_2, H^+ (pH), NH_4^+, H_2S, H_2, and metal ions, the indicator medium of the optodes responding to a change in substance concentrations by changing its optical characteristics such that a chang in fluorescence intensitiy from an inert fluorescing component of the sensor is measured.

In particular, the invention provides, for example, for the testing of blood samples, that the CO_2 concentration be measured continuously and the detection of microorganisms be determined upon a rise of the CO_2 concentration. The invention further provides for using any combination of sensors to identify the microorganisms completely or partially.

In accordance with another variation, the invention will also permit continuous measuring of the O_2 concentration and determination of the changes in H_2S, NH_4 and pH. The advantage of this variation is that the significant changes in the O_2, NH_4, H_2S concentration and pH may be detected earlier than the detection of changes of CO_2 alone.

The invention also provides that a culture medium containing a carbon compound be introduced into a sealable container, and that the sensor be added to the culture medium, which responds to the changes in metabolic substances content by a change in its fluorescence behaviour, and that a blood sample be introduced into the container, which may be subject to metabolic processes in the presence of microorganisms, during which metabolic processes occur, and that the content of the container be exposed to excitation radiation and the radiation emitted by the fluorescent component of the sensor be measured, a change in fluorescence behavior indicating the presence of microorganisms. For example, indicator capsules as disclosed in the German application 23 63 84 may be added, or rather, microcapsules containing sensors, whose walls are made from polymerized hydrophilic monomers.

According to the invention, a device is provided for detecting biological activities in a specimen, comprising a sealable container containing a culture medium into which the sample is introduced, and further comprising means enabling metabolic processes to take place in the presence of microorganisms in the sample, and is characterized by the use of several optodes for simultaneous assessment of several substances whose concentrations are subject to changes by the metabolic processes, and by assigning an excitation and detection assembly to each optode, which, in turn, is connected with an evaluation unit for determining the change over time of the substance concentrations. Combining two optodes (e.g. O_2 and pH) to form a bisensor, or three optodes (e.g. CO_2, O_2, pH) to form a trisensor may be of advantage.

At this point, a description shall be provided with respect to the concepts, types of structure on a system and component basis, technology, principles of the invention and model systems for particular materials, all included in what is referred to as Fluorescence Intensity Modulation Sensor Technology (FIMST).

The present invention is based on certain fundamental concepts including the desire to use an inert fluorescent material in combination with an aqueous suspension of an indicator to provide significant benefits due to:

1. Selection of a high quantum efficiency fluorophore;
2. Selection of fluorophore that has an absorbance match to an inexpensive and powerful excitation

source;

3. Selection of an indicator that is most sensitive to the region of interest;

4. Selection of an indicator with a strong absorbance at either, or both, the excitation or emission wavelength of the fluorophore; and

5. Th potential to use the same optical system (source and detector) for sensors detecting different analytes.

The structure of embodiments of the invention may utilize various components and variations including:

1. Membrane (e.g. gas permeable silicone)
   a) single membrane containing both the fluorescent material and the indicator, or
   b) a multi-layer structure with the fluorophore and indicator in separate layers;
2. Fluorescent material, fluorophore
   a) contained in the silicone, or
   b) contained in an aqueous emulsion;
3. Indicator
   a) contained in the aqueous emulsion,
   b) contained in the sample solution, or
   c) contained in the sensor matrix;
4. Source
   a) light emitting diodes (LEDs) including
      1. blue 456 nm peak or 470 nm peak,
      2. green 560 nm peak, 550--580 nm half intensity,
      3. yellow 590 nm peak, 570---610 nm half intensity,
      4. high intensity red (red/orange) 645 nm peak, 615--660 nm half intensity and
      5. red 665 nm peak, 650--670 nm half intensity; and
   b) other lamps such as tungsten, quartz halogen and neon; and
5. Detector
   a) silicon photodiodes,
   b) PIN silicon diodes,
   c) GaAsP photodiodes, and
   d) other photodetectors including photovoltaics, photoresistive devices and photoconductive devices.

The method diclosed herein has general applicability for the determination of any substance for which a colorimetric method and a permeable membrane can be prepared. Various principles may be used in selecting a sensor system and model sensor systems are discussed hereinbelow.

According to the proncipies of the invention, current optical sensors using fluorescence or absorbance-based materials have inherent limitations. The absorbance and fluorescence-based systems must use different optical systems (light source, filters and detectors) for each sensor system, depending on the optical change of the indicator medium. This is due to the necessity of providing and measuring different wavelengths of light for each different optical system.

The disadvantage for direct fluorescence detection of the metabolic substances of microorganisms is that the source and detectors for use in fluorescence are expensive and of limited availability. In addition, direct fluorescence systems have not yet been described for some tests of interest (e.g., hydrogen sulfide and ammonia).

A system constructed in accordance with the present invention has a sensor and an optical system that provides for the determination of a fluorescent intensity from the sensor. The optical unit has a light source that provides light of the appropriate wavelengths to excite the fluorescent material in the sensor. The light source may be filtered to provide light only at the wavelengths appropriate to the fluorophore. In the most simple case, the source is a light-emitting diode that provides transmission only at the wavelengths needed for excitation.

The detector can be any device that will provide an electronic signal proportional to the light intensity. The preferred embodiment uses a silicon photodiode with a filter to select only the fluorescent wavelength. In a preferred variation, the source and detector are held in a mount that positions the light from the lamp to illuminate the sensor directly above the photodetector. Appropriate optical filters are used to optimize the signal change associated with th optical change in the indicator medium.

The sensor is composed of two parts, an inert fluorescent material which provides the fundamental optical signal and, the indicator material. In practical applications, the sensor is placed and attached to a transparent surface inside a sealable container that can be filled with liquid media that supports the growth of microorganisms.

Th fluorescent material is selected to provide an optical signal matched with the source and detector.

The quantum efficiency of the fluorescent material will, to an extent, determine the detection range of the sensor system. The fluorescent material may be mixed in the sensor with the indicator material, incorporated as a part of the sensor matrix, applied as a coating to the back of the sensor or in any manner in which the light from the source will pass through the indicator before or after passing through the indicator material. The indicator material is selected to provide an optical change in response to the analyte at the concentrations expected. The sensitivity of the sensor system is determined by the amount which the color changes of the indicator interacts with (modulates) the excitation and/or emission of the fluorescent signal. In use, the sensor system provides a constant signal when the concentration of the analyte remains constant. When the analyte concentration changes, the indicator responds by exhibiting a change in optical properties (e.g., color intensity). This change acts as an optical filter to change the amount of light exciting or emitted from the fluorescent substrate. This change in light is detected as an amplitude change (modulation) by the appropriately-filtered photodetector.

A number of different configurations are possible in this sensor system including having separate layers of silicone membrane for the fluorescent material and the indicator. In a preferred variation, the optode is covered with a dye-impregnated silicone to maintain optical isolation for the system. Examples of the construction of the sensor are presented in Tables I, II and III. An example of using this optical system for the detection of carbon dioxide, hydrogen sulfide and pH are presented. In these models systems, the optical unit remains the same even though the indicator component changes.

Several model system examples will now be provided.

The optical system may comprise a yellow LED source and an output of 63 mcd at a peak wavelength of 590 nm. The detector is a silicon photodiode with a peak sensitivity at 720 nm and 0.37 W/A sensitivity at 660 nm.

A model system for the detection of CO_2 is disclosed below. The fluorescent material carboxynaphthofluorescein is suspended in a matrix of carbon dioxide-permeable silicone polymer. Included in this matrix is an aqueous suspension of Bromthymol Blue indicator. The fluorescent material is excited by using the yellow LED with a peak emission of 590 nm and a bandwidth of 40 nm. This corresponds well to the peak absorbance for the fluorescent material carboxynaphthofluorescein at 598 nm. The emission of the fluorescent material is maximum at 660 nm. The sensor is in equilibrium with the carbon dioxide present in the media and this results in the aqueous emulsion of the sensor having a pH of greater than 7.6. If bacteria are present in the sample, the CO_2 produced thereby diffuses through the silicone membrane into the aqueous emulsion and reduces the pH of the emulsion. As the pH is reduced, more of the Bromthymol Blue indicator is converted to the acid form and the color of the emulsion changes from blue to yellow (e.g., peak absorbance changes from 617 nm to 470 nm and the yellow light is not strongly filtered out).

When the indicator is in the base form (blue) it filters out the excitation light and no fluorescent signal is detected. As the pH and the emulsion decreases and more of the base form is converted to the yellow acid form, and more yellow light is allowed to reach the fluorescent material and an optical signal is detected. The light intensity is found to increase with an increase in CO_2 concentration. Experimentally, however, the operational amplifier inverts the signal and in Fig. 3 the voltage signal is decreased with an increase in carbon dioxide.

A model system for the detection of hydrogen sulfide is set forth below. This system acts as a probe, that is, it responds to the presence of H_2S in an irreversible manner. For microorganism detection, the system has a constant high level of signal in the absence of H_2S. The detection of the presence of H_2S will be indicative of a particular group of microorganisms.

The system comprises a fluorescent base material, carboxynaphthofluorescein, suspended in a hydrogen sulfide-permeable silicone polymer. Included in the silicone matrix is a dilute aqueous emulsion of lead acetate. The sensor is illuminated by the yellow LED. The resultant fluorescent signal is detected by the silicon photodiode. In use, the probe provides a constant signal unless there are microorganisms in the sample container that produce hydrogen sulfide. The hydrogen sulfide is soluble in the liquid media and will diffuse into the silicone membrane. Once inside , the hydrogen sulfide will diffuse into the aqueous emulsion containing the colorless lead acetate solution. The hydrogen sulfide reacts with the aqueous lead acetate to produce an insoluble lead sulfide.

The lead sulfide precipitate is black in color and strongly absorbs and scatters both the excitation and emission light. This results in a decrease in the fluorescent signal at the photodiode detector. Th signal will be proportional to the amount of hydrogen sulfide produced until the available lead acetate is completely reacted.

A model sensor system for pH measurement uses the same Bromthymol Blue indicator as does the carbon dioxide sensor. However, instead of having the gas-permeable silicone membrane, this sensor has the indicator immobilized on a support and protected by a hydrogen ion-permeable membrane. As the pH

of the media changes, the ratio of Bromthymol Blue in the acid to base form changes in a manner proportional to the hydrogen ion concentration. This change in color provides a change in the fluorescent signal that is also proportional to the hydrogen ion concentration. This value can be used to determine the pH, as pH = -log (hydrogen ion concentration). The range of this sensor is limited to the range in color change of Bromthymol Blue, 6.2 to 7.6 pH. This range, however, covers the physiological pH range. Should an increase in the range be required, there are other sensor systems listed herein that cover the range of pH from 4.7 to 8.0.

TABLE I

Blue LED (470 nm)

Fluorophores:
1-Acetoxypyren 3,6,8-trisulfonic acid
DCM 4-dicyanomethylene2methyl6(p-dimethylaminostyrol) 4H-pyran

Indicators:	Color-Change	Direction of light-intensity with CO_2 increase
p-Nitrophenol	C-Y	increase
m-Dinitrobenzoyleneurea	C-Y	increase
Azolitmin	R-B	decrease
Bromxylenol Blue	Y-G-B	decrease

Green LED (560 nm)

Fluorophores:
7-Aminoactinomycin D
Nile Red
Rhodamine B

Indicators:	Color-Change	Direction of light-intensity with CO_2 increase
3.6-Dihydroxy xanthone	C-B	increase
Cleves Acid	C-G	increase
Propyl Red	R-Y	decrease
Neutral Red	R-Y	decrease
Bromocresol Purple	Y-G-P	increase
Alizarin	Y-R	increase

Yellow LED (590 nm)

Fluorophores:
Thionin
3.3-Dimethyloxadicarbocyanine
Carboxynaphtho Fluorescein
Naphtho Fluorescein
Sulforhodamine 101

Indicators:	Color-Change	Direction of light-intensity with CO_2 increase
Cleves Acid	C-G	increase
Orcinaurine	C-G	increase
p-Nitrophenol	C-Y	increase
3.6-Dihydroxy xanthone	C-B	increase
Bromxylenol Blue	Y-G-B	increase
Bromthymol Blue	Y-B	increase

Red LED (645 nm)

Fluorophores:
3.3-Diethylthiadicarbocyanine
Nile Blue

Indicators:	Color-Change	Direction of light-intensity with CO_2 increase
Cleves Acid	C-G	increase
Azolitmin	R-B	increase
Bromocresol Purple	Y-G-P	decrease
Alizarin	Y-R	decrease
Propyl Red	R-Y	increase

TABLE II

FLUORESCENT DYES AND PIGMENTS

Abbr.	Dye	Absorbance Max. nm	Fluorescent Max. nm
HPTS	1Hydroxypyren368trisulfonicacid	460	515
APTS	1Acetoxypyren368trisulfonicacid	460	515
RHO-123	Rhodamine 123	505	534
RHO-110	Rhodamine 110	510	535
EO	EOSIN	518	550
7-AAD	7-Aminoactinomycin D	523	647
SFRHO-G	Sulforhodamine G	529	
RHO-6G	Rhodamine 6G Perchlorate	530	556
RHO-6G	Rhodamine 6G Perchlorate	530	590
EVA	Evans Blue	550	610
NIRE	Nile Red Phenoxazon 9	551	636
RHO-B	Rhodamine B	552	580
RHO-B	Rhodamine B	554	627
PYRO	Pyronin B	555	599
SFRHO-B	Sulforhodamine B	556	575
DODC	3.3-Dimethyloxadicarbocyanine	582	660
SFRHO-101	Sulforhodamine 101	586	607
NAPH-FLU	Naphtho Fluorescein	594	663
CARB-FLU	Carboxynaphtho Fluorescein	598	660
TION	Thionin	599	850
NIBL	Nile Blue A Perchlorate	628	690
DTDC	3.3- Diethylthiadicarbocyanine	653	760
DOTCI	Methyl-DOTCI	682	718
IR	IR 144	750	848

TABLE III

ABBR.	INDICATORS	ACID FORM	COLOR CHANGE	BASE FORM
PYR	Propyl Red	4.7	R-Y	6.6
NIPH	p-Nitrophenol	4.7	C-Y	7.9
ACOL	Azolitmin	5.0	R-B	8.0
BROMCRE	Bromocresol Purple	5.2	Y-G-P	6.8
CHLORO	Chlorophenol Red	5.4	Y-R	6.8
DX	3.6-Dihydroxy xanthone	5.4	C-B	7.6
ALI	Alizarin	5.6	Y-R	7.2
BROXBL	Bromxylenol Blue	5.7	Y-G-B	7.5
DPD	3.6-Dihydroxy phthalic dini	5.8	B-G	8.2
NITEU	m-Dinitrobenzoyleneurea	6.0	C-Y	7.8
BROMBL	Bromthymol Blue	6.2	Y-B	7.6
AU	Aurin (Aosolic acid)	6.3	Y-P	6.9
PHENRE	Phenol Red	6.4	Y-O-R	8.0
CLEV	Cleves Acid	6.5	C-G	7.5
ORC	Orcinaurine	6.5	C-G	8.0
RES	Resolic acid	6.8	Y-R	8.0
NEURE	Neutral Red	6.8	R-Y	8.0

pH-Indicators

B-blue	-500 nm
G-green	510-590 nm
Y-yellow	590-620 nm
O-orange	620-640 nm
R-red	640-700 nm
P-purple	700-750 nm
C-colorless	

As indicated above, according to invention, optodes are provided for selecting detection of at least one substance from the group of CO_2, O_2, H_2, H^+ (pH), NH^+_4 metal ions and H_2S, that are present during the metabolic process, as initial, intermediate or the final products.

The excitation and detection assembly may comprise a LED light source and a photodiode detector as well as a two-armed optical waveguide transmitting excitation radiation to the optode or optodes at carrying of the optical signal to the detector.

In a preferred variation of the invention, the optodes are combined to form a multilayer sensor.

In a simple structure of the invention, the optodes are attached to the inside of the wall of a transparent container and are connected to the evaluation unit via the excitation and detection assembly that may be placed flush against the outside of the wall of the container. The optodes, which may be mass produced inexpensively, are attached directly or with an adhesive to the inner surface of the wall of the sample container, which is then filled with the culture medium, sealed and stored. After the addition of the specimen, for example, a blood sample, the container is thermostat-controlled for the time required for the growth of the culture, and is shaken if necessary, whereupon the concentration of the substance subject to chemical reaction by the metabolic processes is measured, for example, with a suitable optically-filt red photodiode in close proximity to the optode.

According to the invention, it is possible to place at least the optodes in the gas space of the at least partially transparent container above the culture medium mixed with the sample, and to use these optodes for measuring the concentration of at least one gaseous metabolite.

It is provided in accordance with the further variation of the invention that the optodes be located on a transparent stopper used for sealing the container.

The method will also permit, however, to place the optodes in a portion of the container covered by the culture medium mixed with the sample, possibly at the bottom of the container, and to use the optodes for measuring the concentration of at least one substance in the culture medium. With this arrangement, the metabolite is measured immediately at the place where it is produced, which will permit more rapid

assessment as to whether a culture is positive or negative.

According to a particularly favorable structure of the invention, there is provided a device for temperature control of the sample, in which several containers ar placed in labeled positions at the same time, each container being assigned an excitation and detection assembly transmitting excitation radiation through the optodes located in each container and detecting the ensuing optical signal, and the signals of the detection assembly are carried to an evaluation unit together with a position indentification signal. The device used for temperature control may be configured as a temperature-controlled supporting rack with multiple optical stations, which will permit a large number of culture models, e.g., up the 600, to be monitored simultaneously. As compared to conventional equipment of this kind, no further handling of the samples is required once they have been filled into their individual bottles, since both the incubating process and the continuous monitoring are fully automated in the device of the present invention. Unlike conventional measuring techniques, in which the individual culture bottles must be inserted into an evaluation unit by hand once or twice daily, the technique of taking measurements continuously will allow the point in time when a culture becomes positive to be determined without delay. One or more substances involved in the metabolic process may be assessed optically and the presence of microorganisms may be determined very quickly. Therefore, a highly-sensitive automatic measuring system permitting noninvasiv , continuous measuring techniques is provided by the invention. The evaluation unit either includes a microcomputer/microcontroller indicating the status of each individual container, or it is connected to a computer via an interface. Information useful for bacterial identification would be available with multiple optodes.

In another structure of the invention, provisions are made for a device for temperature control of the sample, which holds several containers at the same time, and for a feed mechanism or sample changer automatically taking the individual containers to a measuring station, in which the optodes located in each container enter into optical contact with the excitation and detection assembly. Whereas the variation described in the foregoing paragraph has no moveable parts at all, this structure has a conventional sample changer for automatically taking each sample to a measuring station. The advantage of this arrangement is that the electronic or electro-optical equipment need not be so elaborate.

According to the invention, it is also possible to fasten the optodes at the tip of the probe to be inserted into the container, which probe contains light waveguide elements from the excitation and detection assembly. The probe may be inserted through an opening sealed by a septum and introduced into the culture medium mixed with the sample or into the gas space thereabove.

A further variation of the invention provides that the container be sealed by a septum which may be punctured with a hollow needle of a sampling vessel, the optodes being located in the sampling vessel and a flow connection being established between the gas space of the container and the optodes via the hollow needle of the sampling vessel, after the sample has been added to the culture medium in the container. As a sampling vessel an evacuated vessel may be used, for example, to whose inner wall surface optodes may be affixed. The sample, e.g., blood, is drawn into the container by the vacuum applied to the vessel. Th septum of the container holding the culture medium is pierced with the needle and the blood is introduced into the culture bottle. In view of the hollow needle, metabolic gases are conveyed from the gas space above the culture medium to the sensor, where they are measured. The culture bottle and sampling vessel are preferably designed as single-use articles that are discarded after use.

Other objects, features and advantages of the invention, its organization, construction and operation will be best understood from the following detailed description, taken in conjunction with the accompanying drawings, on which:

Fig. 1 is a schematic representation of a device for practicing the invention;

Figs. 2a, 2b, 2c, 2d, 3 and 4 are similar views of variations of the device of Fig. 1;

Fig. 5 is a schematic representation of a variation for automatic measuring of several containers at the same time;

Figs. 6 and 7 are schematic representations of variations of the structure of Fig. 5;

Figs. 8, 9 and 10 are graphic illustrations of curves of measured concentrations; and

Fig. 11 is a graphic illustration of measured curves using the sensor of the present invention to detect the growth of a variety of bacteria commonly found in cases of bacteremia.

The d vice of Fig. 1 for detecting biological activities in a sample comprises a sealable, optically-transparent container 1 with an optode 3 attached to the inner surface 2 of its wall and bonded by a transparent adhesive layer 4.

Instead of a single optode 3 for a substance to be assessed, two or more optodes, 3a, 3b and 3c may be combined into a multilayer sensor, which will permit simultaneous detection of the changes in O_2 and CO_2 concentrations and in pH, for example. The individual optodes 3a to 3c or th ir indicator media may be

stacked in layers one above the other, or they may be imbedded in a polymer membrane in homogenous distribution. The combination of a C_2 and an O_2 optode into a sensor is described in the European application 0 105 870, for example.

Instead of the optode 3 in the variations discussed below, optodes may be provided for measuring O_2, CO_2, H^+ (pH), NH_4^+, H_2S and H_2, or rather, a specific combination of these optodes in accordance with the particular requirements.

The container 1 contains the culture medium 4 with one carbon compound (glucose), for example, which is converted by metabolic processes of microorganisms in the sample and to metabolic product, for example CO_2, during which processes O_2 is being consumed and the pH is subject to change. As a consequence, there are changes in the concentration of the metabolic product and the initial substances and the gas space 6 above the culture medium 5 and in the culture medium itself, which are detected by way of the optodes 3a, 3b and 3c placed at the bottom 7 of the container 1 in Fig. 1. The excitation and detection assembly 8 comprises a light source 9, a detector 11 and a two-armed light waveguide 10, one of whose arms is coupled to the light source 9 and the other of which is coupled to the detector 11. The end 12 of the light waveguide is placed flush against the exterior 13 of the wall of the container, transmitting excitation radiation towards the optodes 3a, 3b and 3c through the transparent wall of the container, while receiving the optical signal, e.g., the fluorescence radiation emitted by the optodes.

The use of a suitable filter 31, for example, a filter disc, at front of the detector 11 will ensure that the signals are assigned to their corresponding optodes 3a, 3b, 3c.

By way of a line 14, the detector signals are transmitted to an evaluation unit 15 in which the change over time, e.g., of the CO_2 content is determined and the status of the sample is indicated via a display 16.

The conditions in the container necessary for the metabolic processes are maintained by way of the unit 17, which is mainly responsible for proper temperature control of the sample, and is connected with the evaluation unit 15 via a control lead 18.

Instead of being a 17, an air heating element may be used for sample temperature control of the variation of Fig. 1 and all subsequent variations.

The structure illustrated in Fig. 2a differs from that of Fig. 1 only by the fact that the optode 3 is located in the gas space 6 of the container 1 and that the metabolites may be measured only. In this instance, temperature control is performed via the bottom 7 of the container 1. In a structure according to Fig. 2b, the optode 3, or the optodes 3a, 3b, may be attached to a stopper 1' sealing the container 1. The light waveguide 10 may go either through this stopper 1' or it may be placed on the exterior of the transparent stopper 1', as is shown in Fig. 2b. Figs. 2c, 2d and 2e illustrate other modifications of Fig. 1 which utilize LEDs and photodiode detectors even though each optode has a different chemistry and produces a different optical change (e.g., color). The sensor's optical system is being used because of the fluorescence is being measured.

In another structure as presented in Fig. 3, the optode 3 is attached to the tip of a probe 19 receiving the end of the two-armed light waveguide 10. The probe 19 is introduced into the container 1 through the opening 21 sealed by a septum 20, and may be axially shifted along in the direction of the arrow 22, permitting measurements to be taken both in the gas space 6 and in the culture medium 5.

In the structure illustrated in Fig. 4, the optode 3, which is used, for example, for measuring O_2 and CO_2, is not located in the container 1, but is placed in a sampling vessel 23. In order to introduce the sample into the culture bottle, the septum 20 of the container 1 is punctured with the hollow needle 24 of the sampling vessel 23, permitting the sample to enter the culture medium. Via the hollow needle 24 a gas exchange will take place between the gas space 6 and the interior 25 of the sampling vessel 23 such that the change in the concentration of CO_2 and O_2 may be determined with the use of the excitation and detection assembly 8 which is symbolically indicated by the light waveguide 10.

A particularly favorable structure is illustrated in Fig. 5 comprising a device 26 configured as a temperature-controlled supporting rack, which will hold several containers 1 at the same time. The containers are inserted in labeled positions and are arranged in several rows, permitting temperature control and continuous monitoring of up to 600 containers simultaneously. Each container is assigned a two-armed light waveguide 10 located in the supporting rack 26 and providing the optodes 3a to 3c at the bottom of each container 1 with excitation radiation. The corresponding optical signals are delivered to the individual detectors 11 connected to the evaluation unit 15 via lines 14. The individual readings delivered to the evaluation unit 15 are accompained by suitable position identification signals such that the individual values may be directly assigned to the corresponding sample.

A structure as presented in Fig. 6 provides that each individual contain r 1 be connected with a LED 27 located in the supporting rack 20 and with a photodiode 28, possibly in conjunction with photo elements. In this manner, a most compact device is obtained which is characterized by the total absence of moveable

parts.

Fig. 7 illustates a structure according to Fig. 6, which contains two optodes, 3a and 3b, combined into a sensor (.g., a bis nsor), for simultaneous measurement of O_2 concentration and pH. The optodes are excited via different LEDs 27 and 27' whose emission radiation is received by a common photodiode 28. The corresponding electrical leads 29, 29', 30 lead to the evaluation unit not shown here. By means of known optical or electronic equipment, the signals of the two optodes may be separated. Other variations with only one LED for excitation and several photodiodes for signal detection are within the scope of this invention.

In the graphic illustrations of Figs. 8 and 9, the time t, or rather, the individual points in time T_0 to T_8 are plotted on the abscissa, while the pH value, the concentration K (or the partial pressure of O_2 and CO_2) and the number of bacteria or organisms per unit volume (logarithmic scale) are plotted on the ordinate.

Fig. 8 illustrated the change over time of the parameters O_2, CO_2 and pH using a sample containing Staphylococcus areus. Between the times T_5 and T_6 the concentration of CO_2 is charaterized by a significant increase indicating a positive sample, and about the same time the O_2 and pH levels decrease.

As opposed to Fig. 8, the pH value in Fig. 9 remains largely unchanged, whereas the O_2 decrease occurs significantly sooner than the CO_2 increase. In this instance, a sample with Psaudomonas aeruginosa was used.

The values provided in Fig. 10 are taken from a sample containing enterobacteria (E.Coli). All three parameters change and once again the O_2 decrease was noticed prior to the changing of the other parameters.

The method and device described herein are well-suited for detecting biological activities and samples, for example, of microorganisms in blood, (bacteriemia, septicemia or pyemia).

The continuous, noninvasive monitoring of specimens helps to obtain fully automated incubation and measuring processes for a large number of samples. Positive cultures are quickly identified and erroneous negative findings are avoided.

Although we have described our invention by reference to particular illustrative embodiments and examples thereof, many changes and modifications of the invention may become apparent to those skilled in the art without departing from the spirit and scope of the invention. We therefore intend to include within the patent warranted hereon all such changes and modifications as may reasonably and properly be included within the scope of our contribution to the art.

Claims

1. A method for detecting biological activities in a specimen, comprising:

   intoducing a sample of the specimen and a culture medium into a sealable transparent container and sealing the same therein;

   exposing the specimen to conditions enabling metabolic processes to take place in the presence of microorganisms in the sample, the concentration of initial substances being lowered and that of metabolic products being raised during metabolic processes;

   generating a fluorescent signal with a fluorescent sensor in response to changes of concentration of at least one substance subject to conversion by the metabolic processes;

   optically interrogating the fluorescent signal to produce a test signal indicating the change of concentration; and

   monitoring the signal over time for changes indicating the presence of microorganisms.

2. The method of claim 1, wherein the sensor comprises an activatible, inert fluorophore component, and an indicator component which changes its optical characteristics in response to changes in concentration of the at least one substance, and the steps of generating and optically interrogating a fluorescent signal are further defined as:

   modulating the fluorescent emission of the fluorophore component with the optical changes of the indicator to produce the fluorescent signal; and

optically detecting the modulated fluorescent signal.

3. A method for detecting biological activities in a specimen, comprising the steps of:

introducing a sample of the specimen and a culture medium into a sealable transparent container and sealing the same therein along with an optode which comprises an otherwise inert fluorophore and a CO_2-sensitive color indicator;

exposing the sample to condition enabling metabolic processes to take place in the presence of microorganisms in the sample which increase the CO_2 concentration;

exciting the otherwise inert fluorophore through the transparent container with an external light source to cause emission of a fluorescent signal;

modulating the fluorescent signal with the CO_2-sensitive color indicator in response to the changes in CO_2 concentration; and

detecting the modulated fluorescent signal over time to indicate the presence of microorganisms.

4. A method for detecting biological activities in a blood sample, comprising the steps of:

introducing a culture medium containing a carbon compound therein into a sealable transparent container;

adding an inert fluorescent sensor to the culture medium which responds to a change in CO_2 content to change its fluorescent behavior;

sealing the container;

introducing a blood sample into the container;

subjecting the blood sample to metabolic processes in the presence of microorganisms during which CO_2 is produced;

radiating the contents of the container through the wall of the container with radiation of a character that causes emission of a fluorescent signal by the fluorescent sensor, the fluorescent signal being changed by the fluorescent behavior of the indicator in response to the CO_2 content; and

detecting the changing fluorescent signal over a period of time to indicate the presence of the mircroorganisms.

5. Apparatus for detecting biological activities in a specimen, comprising:

a sealable container including a transparent wall section;

a culture medium and a sample of the specimen therein sealed in said container;

means acting on the contents of said container for enabling metabolic processes to take place in which the concentrations of individual substances change;

a plurality of optodes mounted to said transparent wall section and each simultaneously responsive with the others of said optodes to indicate, by fluorescent emission changes, changes in concentration of a respective substance caused by metabolic processes;

illumination means for illuminating said plurality of optodes through said transparent wall section of said container to excite said plurality of optodes to emit the respective fluorescent signals; and

detecting means optically coupled through said transparent wall section of said container to said

plurality of optodes for detecting the changing fluorescent signals.

6. The apparatus of claim 5, wherein:

each of said optodes is responsive to indicate changes in concentration of one substance selected form the group consisting of CO_2, O_2, H^+ (pH), NH_4^+, H_2S, H_2 and metal ions to change its optical characteristics.

7. The apparatus of claim 5, wherein:

at least two of said optodes are combined to form a bisensor for indicating concentration changes of O_2 and pH.

8. The apparatus of claim 5, wherein at least three of said optodes are combined to form a trisensor for indicating concentration changes of CO_2, O_2 and pH.

9. The apparatus of claim 5, wherein one of said optodes is a CO_2 sensor and comprises:

a CO_2-permeable silicone matrix;

carboxynaphthofluorescein fluorescent material suspended in said matrix; and

a Bromthymol Blue aqueous emulsion as an indicator in said matrix.

10. The apparatus of claim 5, wherein one of said optodes is an H_2S sensor and comprises:

an H_2S permeable silicone polymer matrix;

carboxynaphthofluorescein suspended in said matrix; and

a dilute aqueous emulsion of lead acetate as an indicator in said matrix.

11. The apparatus of claim 5, wherein one of said optodes is a pH sensor and comprises:

a support;

an indicator including Bromthymol Blue immobilized on said support; and

a hydrogen ion-permeable membrane covering said indicator.

12. The apparatus of claim 5, wherein each of said optodes comprises:

a fluorophore selected from the group consisting of 1-acetoxypyren, 3,6,8-trisulfonic acid and DCM 4-dicyanomethylene-2-methyl-6(p-dimethylaminostyrol) 4H-pyran.

13. The apparatus of claim 5, wherein each of said optodes comprises:

an indicator selected from the group consisting of p-Nitrophenol, m-Dinitrobenzoyleneurea, Azolitmin and Bromxylenol Blue.

14. The apparatus of claim 5, wherein each of said optodes comprises:

a fluorophore selected from th group consisting of 7-Aminoactinomycin D, Nile Red and Rhodamine B.

15. The apparatus of claim 5, wherein each of said optodes comprises:

a fluorophore selected from the group consisting of Thionin, 3.3-Dimethyloxadicarbocyanine, Carboxynaphthofluorescein and Sulforhodamine 101.

16. The apparatus of claim 5, wherein each of said optodes comprises:

an indicator selected from the group consisting of Cleves Acid, Orcinaurine, p-Nitrophenol, 3.6-Dihydroxy xanthone, Bromxylenol Blue and Bromthymol Blue.

17. The apparatus of claim 5, wherein each of said optodes comprises:

a fluorophore selected from the group consisting of 3.3-Diethylthiadicarbocyanine and Nile Blue.

18. The apparatus of claim 5, wherein each of said optodes comprises:

an indicator selected from the group consisting of Cleves Acid, Azolitmin, Bromocresol Purple, Alizarin and Propyl Red.

19. The apparatus of claim 5, wherein each of said optodes comprises:

a fluorescent material selected from the group consisting of 1-Hydroxypyren-3,6,8-trisulfonicacid, 1-Acetoxypyren-3,6,8-trisulfonicacid, Rhodamine 123, Rhodamine 110, EOSIN, 7-Aminoactinomycin D, Sulforhodamine G, Rhodamine 6G Perchlorate, Evans Blue, Nile Red Phenoxazon 9, Rhodamine B, Pyronin B, Sulforhodamine B, 3.3-Dimethyloxadicarbocyanine, Sulforhodamine 101, Naphtho Fluorescein, Carboxynaphtho Fluorescein, Thionin, Nile Blue A Perchlorate, 3.3-Diehtylthiadicarbocyanine, Methyl-DOTCI and IR 144.

20. The apparatus of claim 5, wherein each of said optodes comprises:

an indicator material selected from the group consisting of Propyl Red, p-Nitrophenol, Azolitmin, Bromocresol Purple, Chlorophenol Red, 3.6-Dihydroxy xanthone, Alizarin, Bromxylenol Blue, 3.6-Dihydroxy phthalic dini, m-Dinitrobenzoyleneurea, Bromthymol Blue, Aurin (Aosolic acid), Phenol Red, Cleves Acid, Orcinaurine, Resolic acid and Neutral Red.

21. A method for detecting biological activities in a specimen, comprising the steps of:

introducing a sample of the specimen and a culture medium into a sealable transparent container along with an inert, activatable fluorophore which is responsive to radiation within a predetermined wavelength band to emit a fluorescent signal in accordance with the intensity of radiation, and at least one optical indicator which is responsive to the concentration of a specific substance to change its optical characteristics, and sealing the same in the container;

exposing the contents of the container to conditions enabling metabolic processes to take place in the presence of microorganisms in the sample, the concentration of inital substances being lowered and that of metabolic product substances being raised during the metabolic processes;

applying radiation within the predetermined wavelength band to the contents of the container;

generating and emitting a fluorescent signal with the fluorophore in accordance with the intensity of the radiation;

modulating the intensity of the fluorescent signal emitted by the fluorophore with the at least one optical indicator in accordance with the optical characteristics of the at least one optical indicator established by the concentration of the specific substance due to conversion by the metabolic processes; and

monitoring the modulated fluorescent signal over time for changes indicating the presence of microorganisms.

22. A method for detecting biological activities in a specimen, comprising the steps of:

introducing a sample of the specimen and a culture medium into a sealable transparent container along with an inert activatable fluorophore which is responsive to radiation within a predetermined wavelength

band to emit a fluorescent signal in accordance with the intensity of radiation thereon, and at least one optical indicator which is responsive to the concentration of a specific substance to change its optical characteristics, and sealing the same in the container;

exposing the contens of the container to conditions enabling metabloic proc sses to take place in the presence of microorganisms in the sample, the concentration of initial substances being lowered and that of metabolic product substances being raised during the metabolic processes;

applying radiation within the predetermined wavelength band to the contents of the container;

modulating the radiaton, prior to it reaching the fluorophore, with the at least one optical indicator in response to the optical characteristics of the at least one optical indicator established by the concentration of the specific substance due to conversion by the metabolic processes;

generating a fluorescent signal with the fluorophore in accordance with the intensity of the modulated radiation; and

monitoring the fluorescent signal over time for changes indicating the presence of microorganisms.

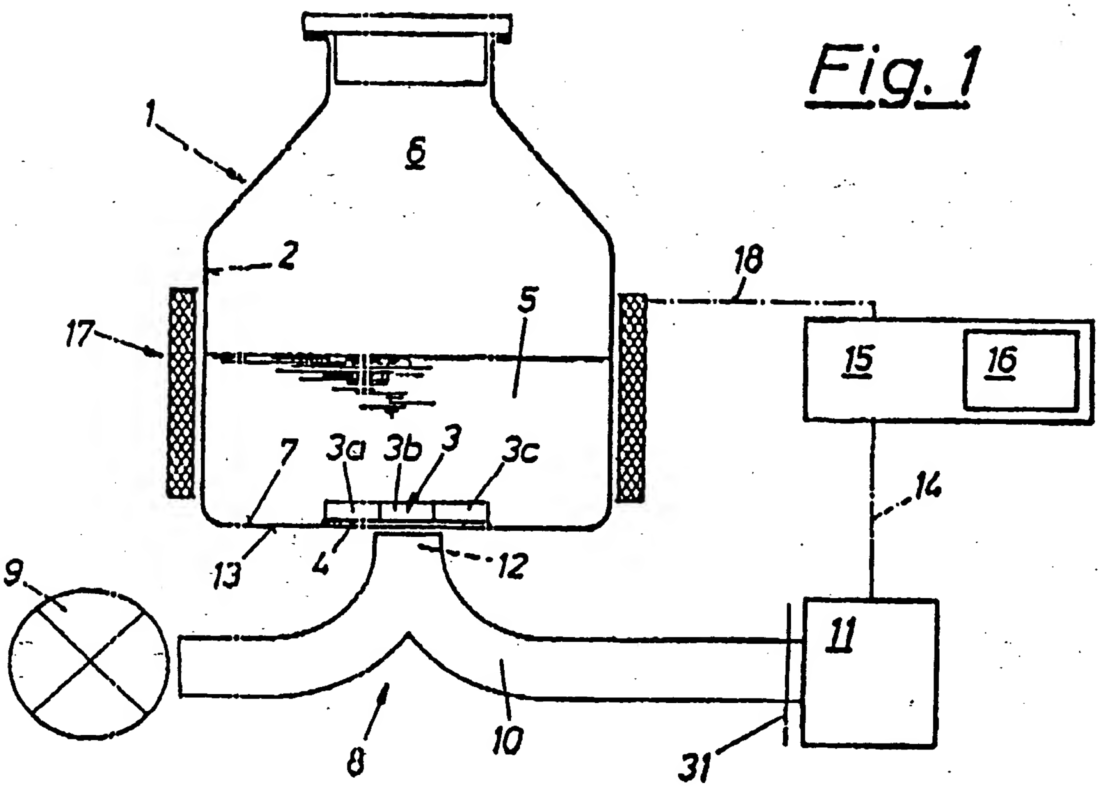

Fig. 1

Fig.2a

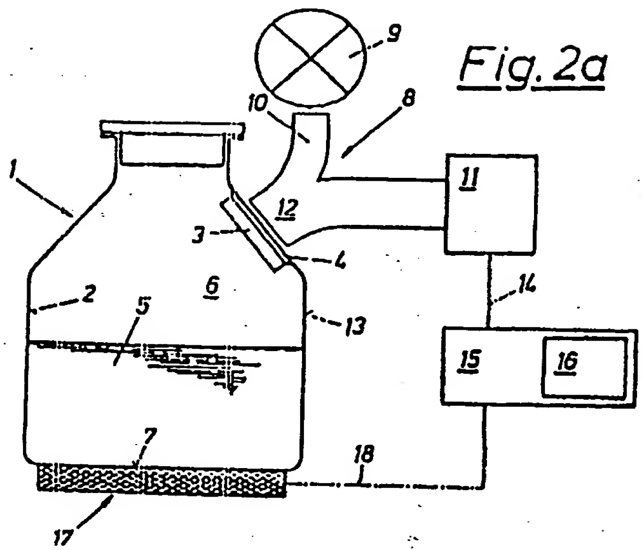
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Fig. 2c

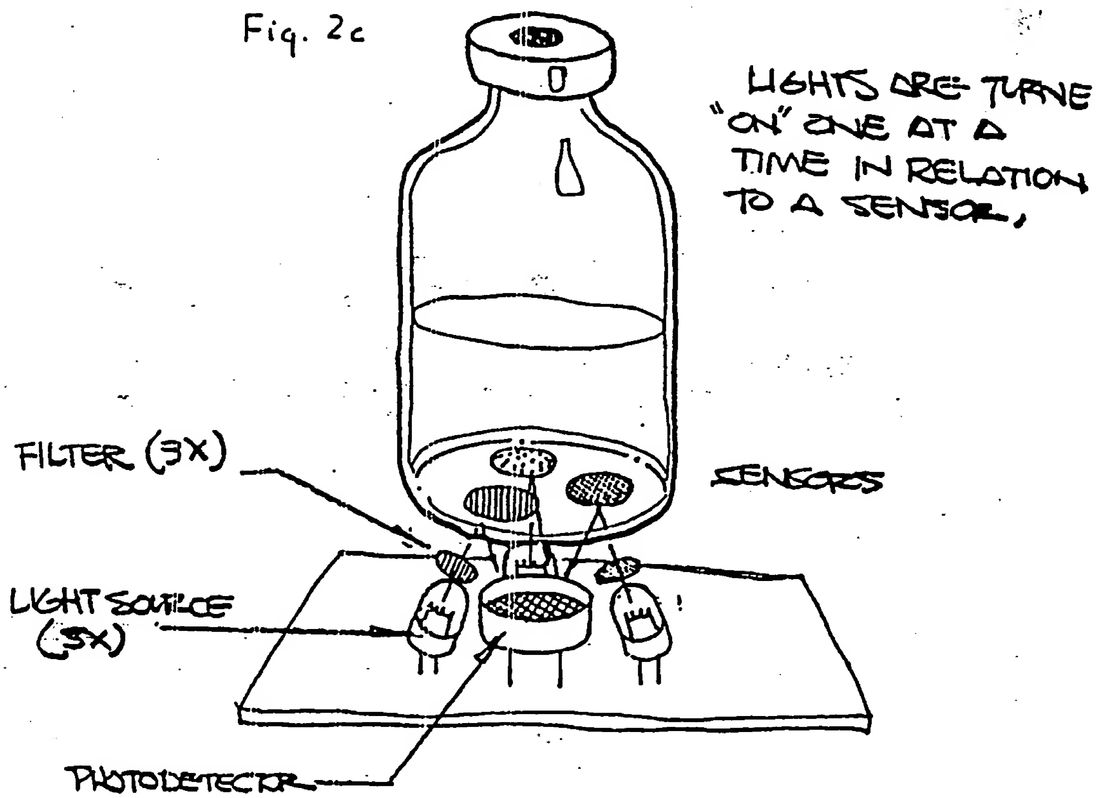

LIGHTS ARE TURNE "ON" ONE AT A TIME IN RELATION TO A SENSOR,
FILTER (3X)
SENSORS
LIGHT SOURCE (3X)
PHOTODETECTOR

Fig. 2d

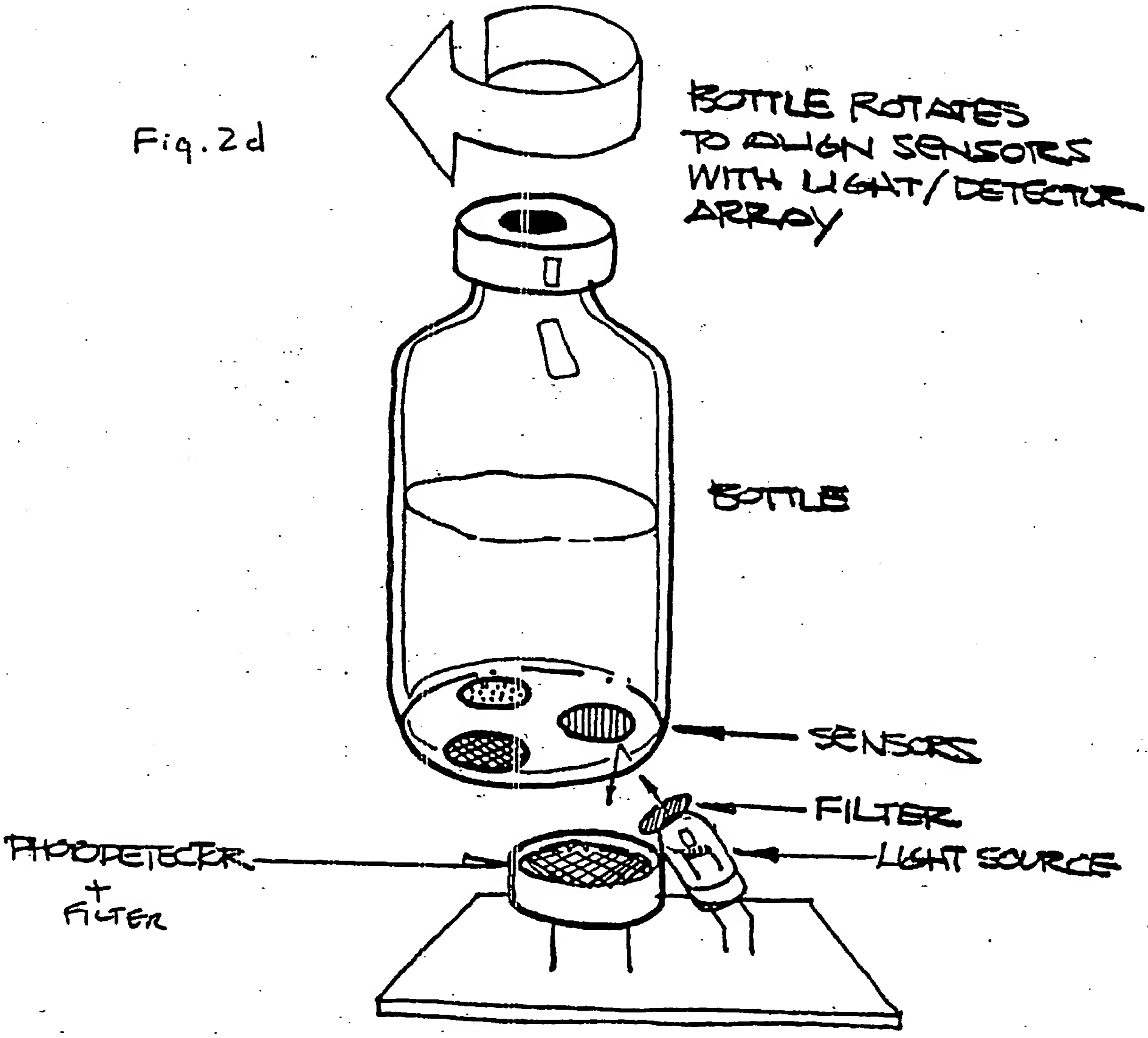

BOTTLE ROTATES
TO ALIGN SENSORS
WITH LIGHT/DETECTOR
ARRAY
BOTTLE
SENSORS
FILTER
PHOTODETECTOR
+
FILTER
LIGHT SOURCE

Fig. 2e

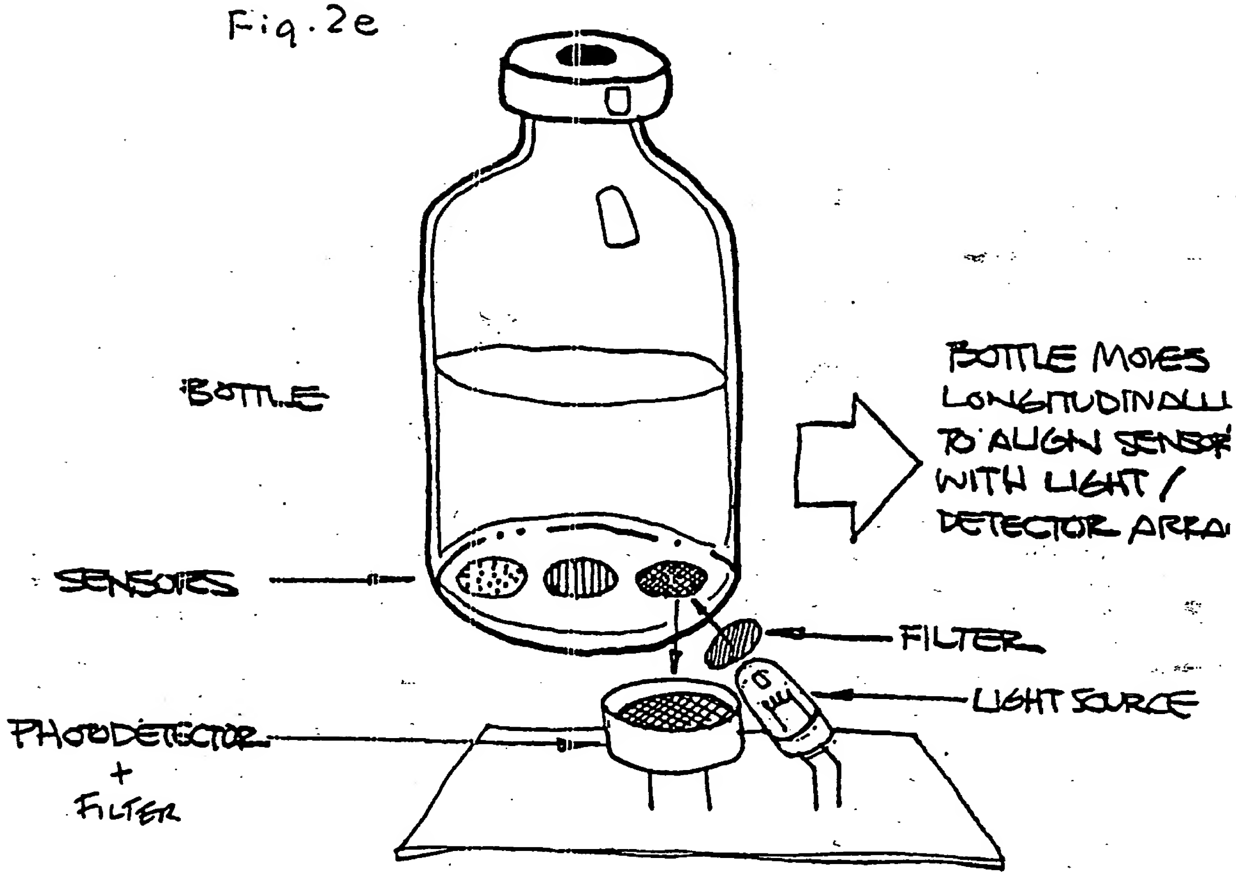
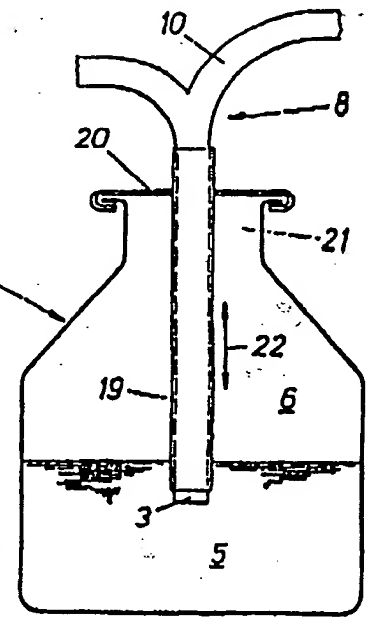

BOTTLE
BOTTLE MOVES LONGITUDINALLY TO ALIGN SENSOR WITH LIGHT / DETECTOR ARRA
SENSORS
FILTER
LIGHT SOURCE
PHOTODETECTOR + FILTER

Fig. 3



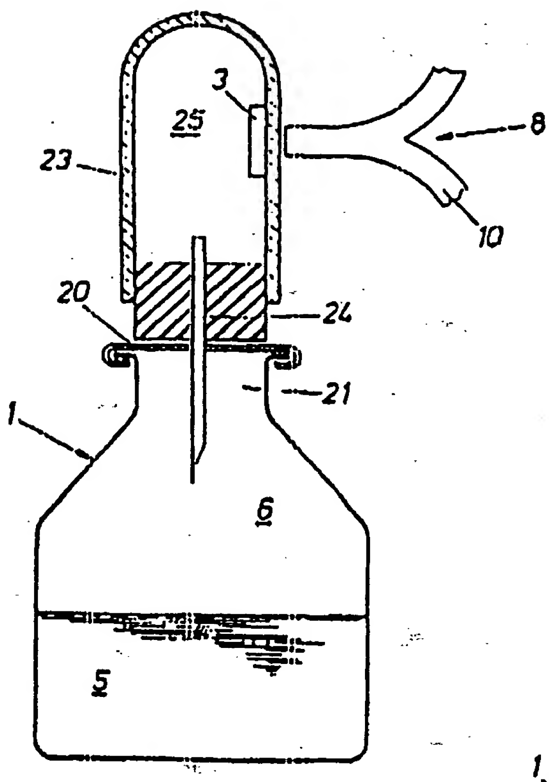


Fig. 4

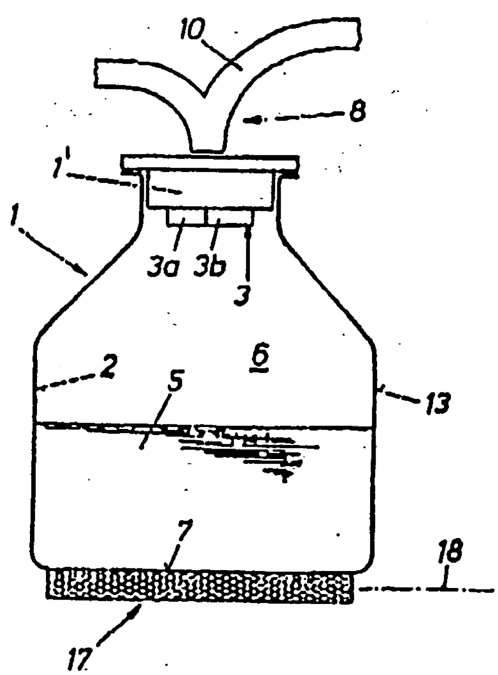


Fig. 2b

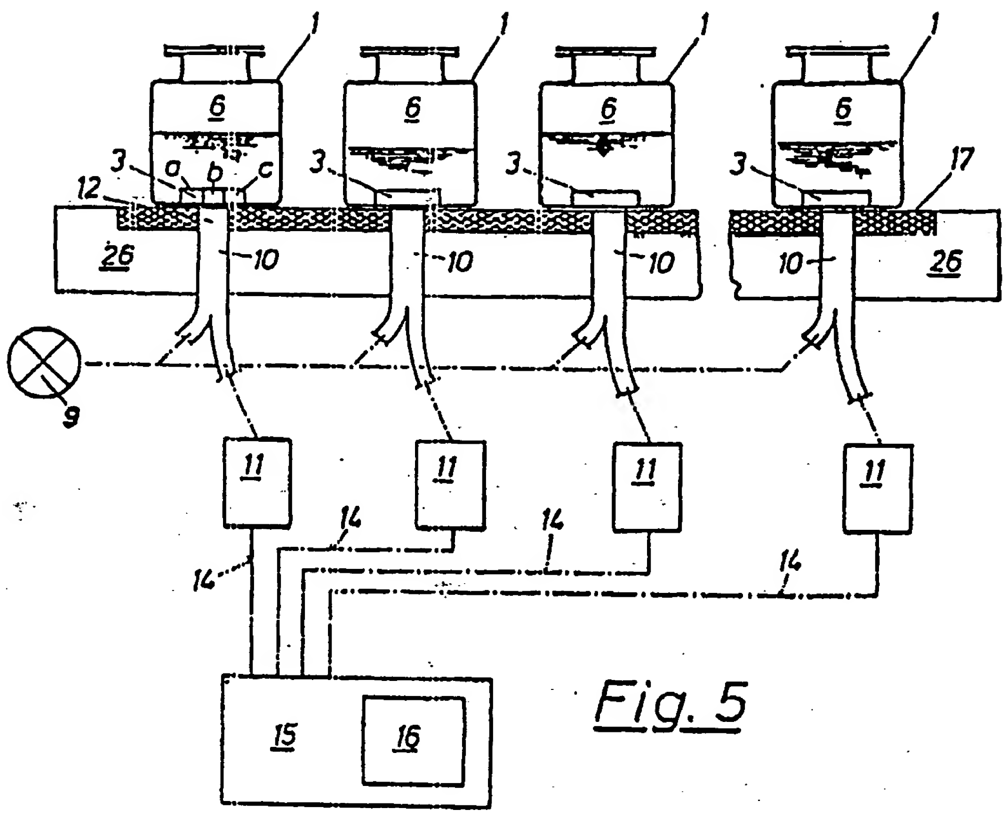


Fig. 5

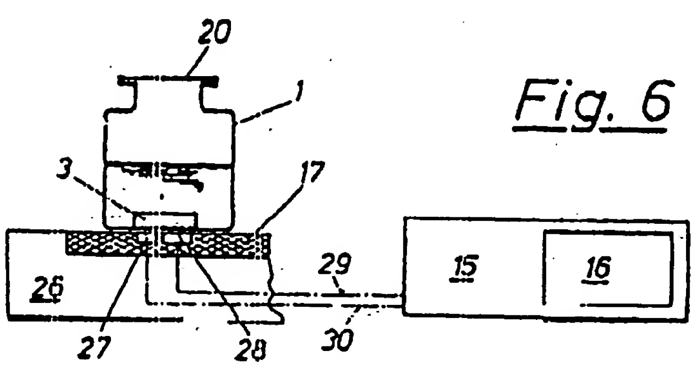


Fig. 6

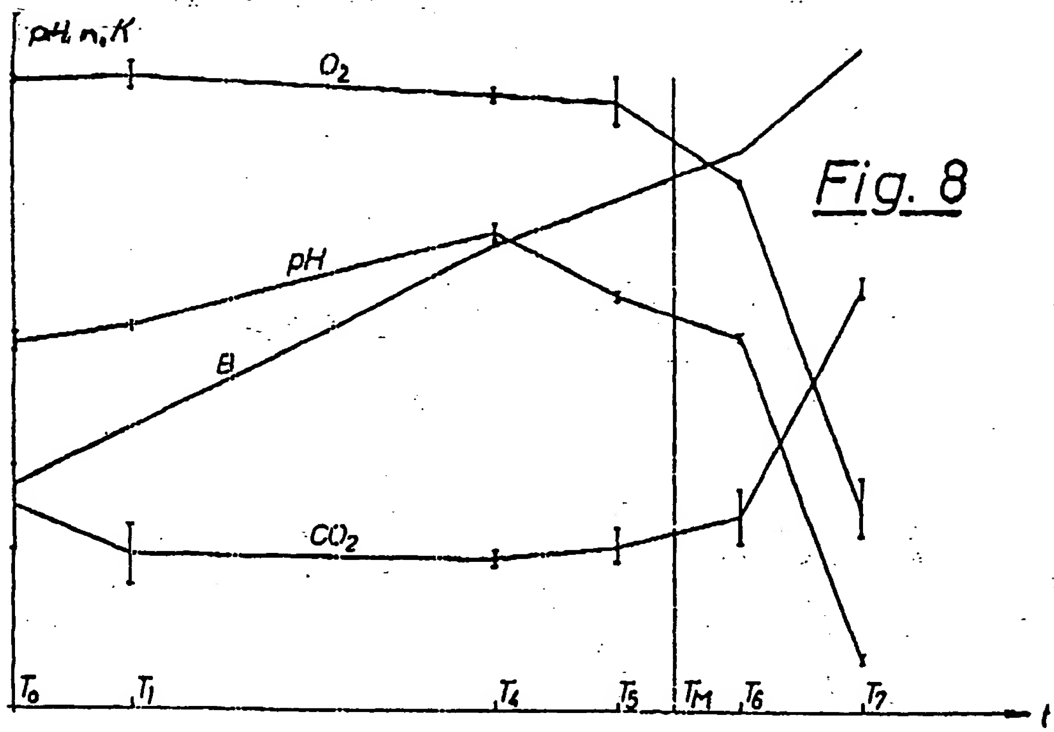

pH, n, K
O2
pH
B
CO2
T0
T1
T4
T5
TM
T6
T7
t

Fig. 8

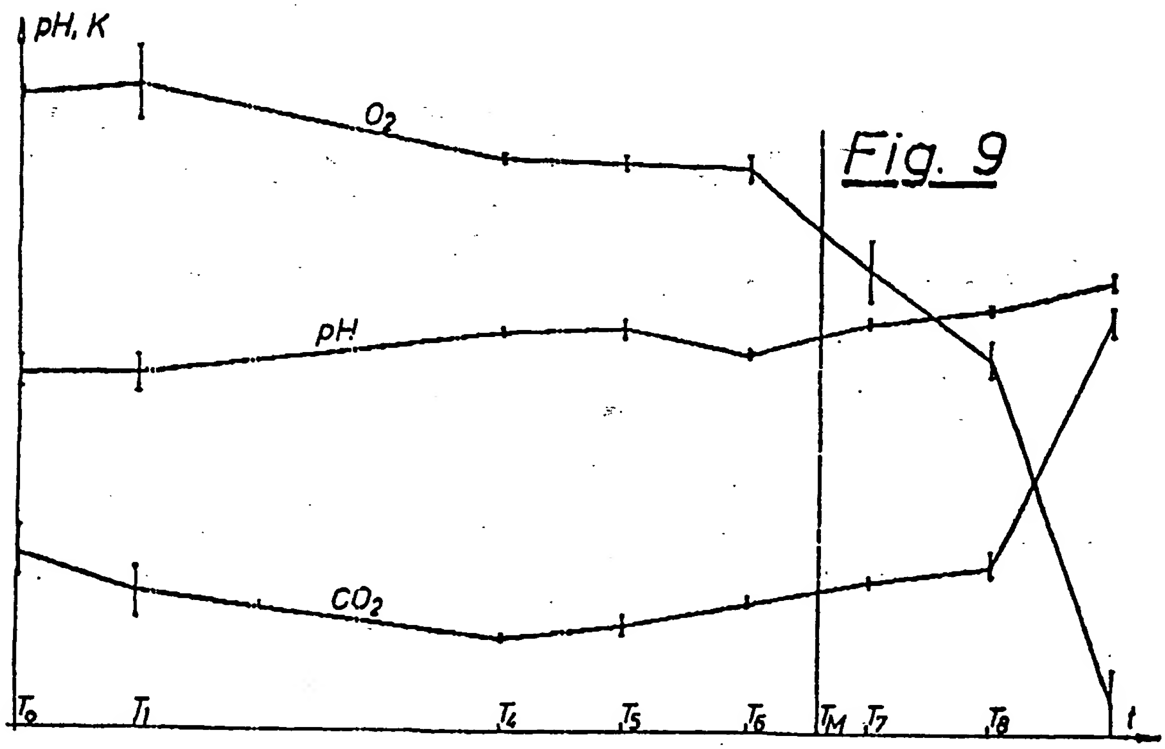

pH, K
O2
pH
CO2
T0
T1
T4
T5
T6
TM
T7
T8
t

Fig. 9

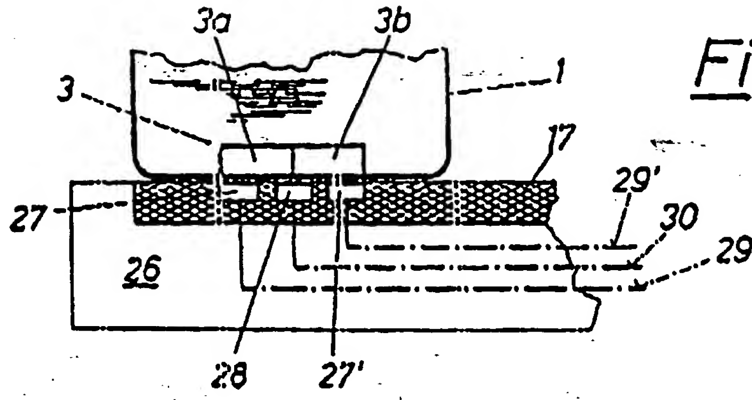


Fig. 7

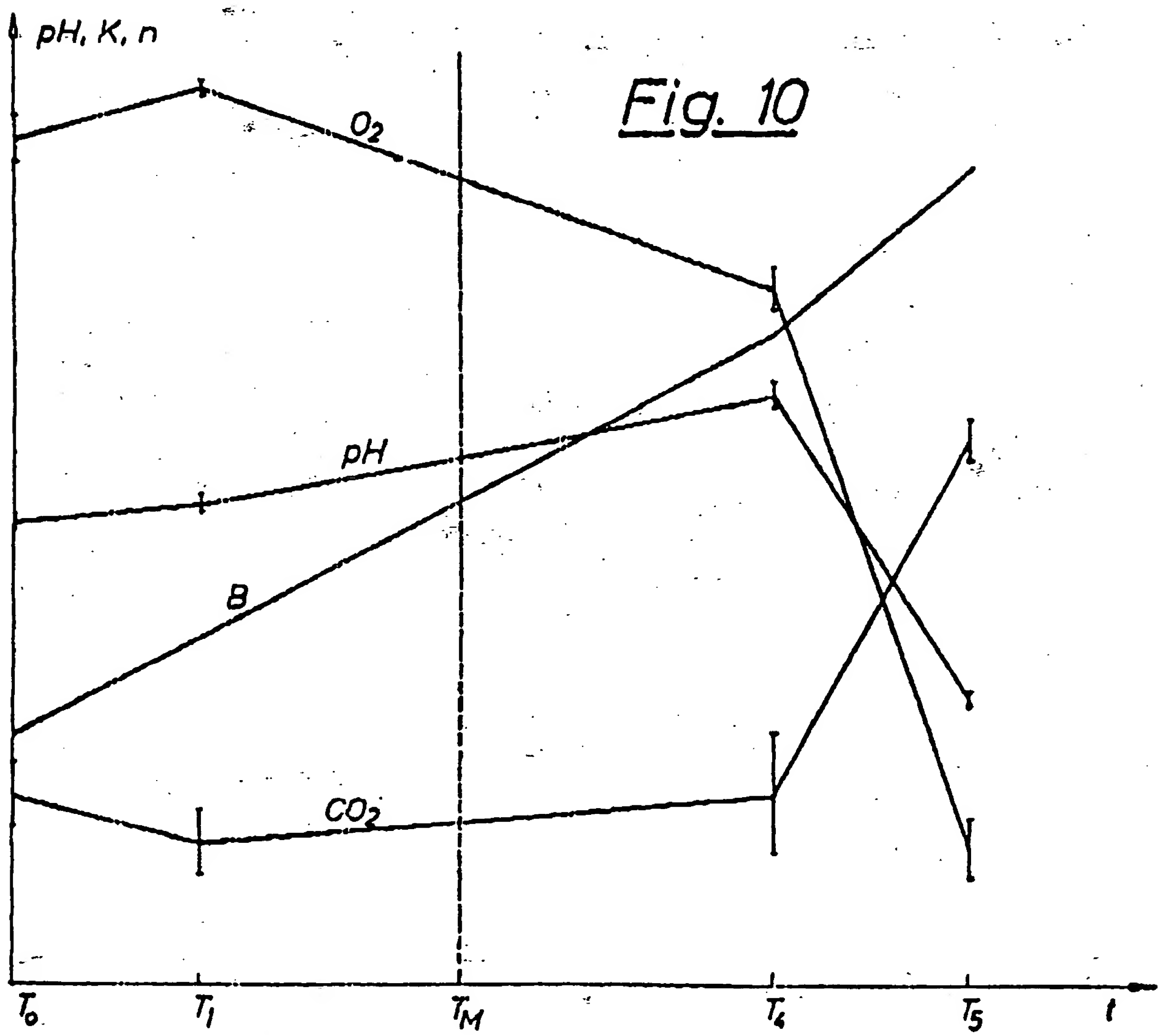
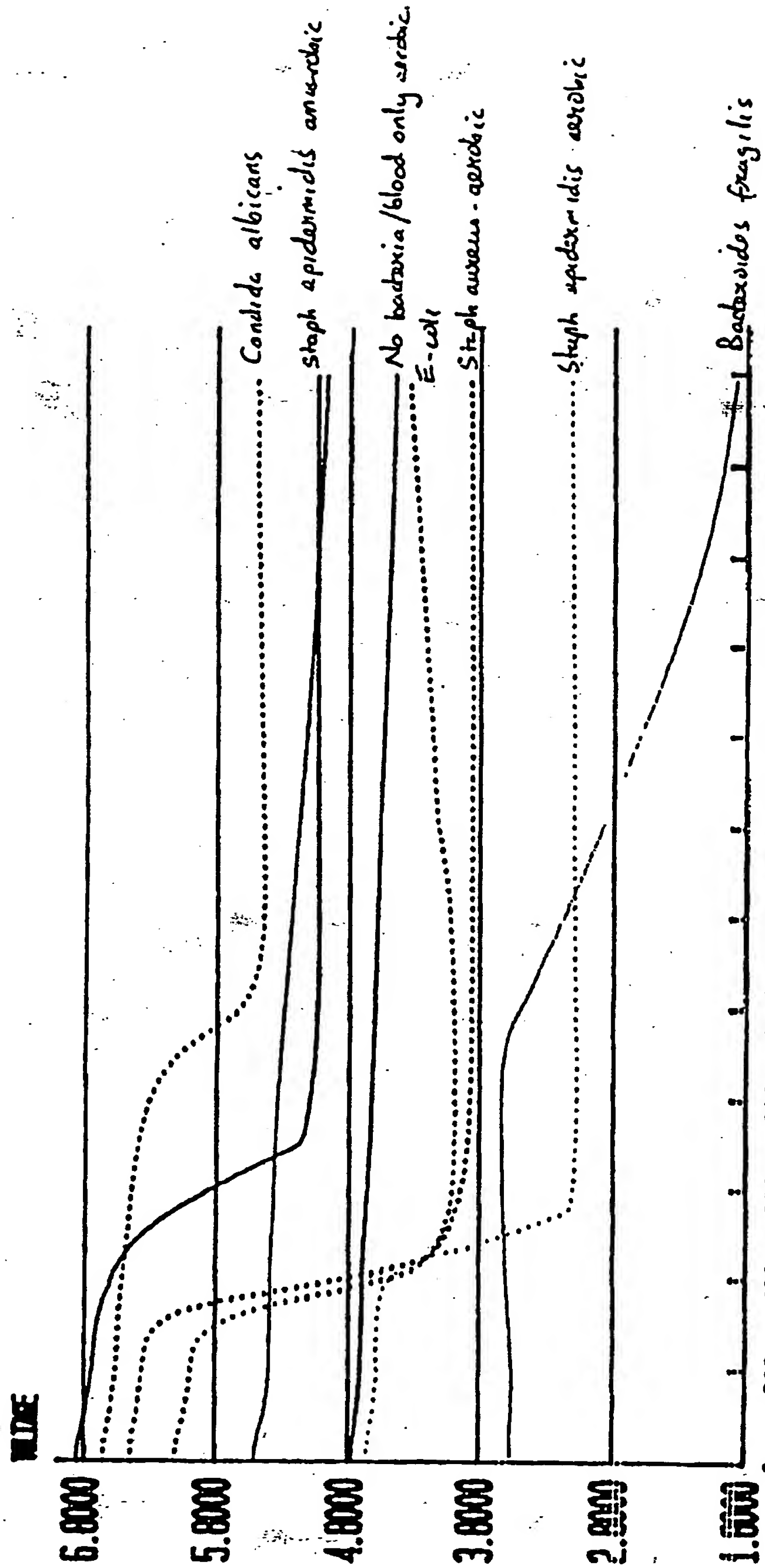


Fig. 10

Fig 11


Candida albicans
Staph epidermidis anaerobic
No bacteria/blood only aerobic
E-coli
Staph aureus - aerobic
Staph epidermidis aerobic
Bacteroides fragilis
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MINUTES

European Patent Office

EUROPEAN SEARCH REPORT

Application Number

EP 90 89 0304

DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int. Cl.5)
D,X D,Y D,A	EP-A-0 333 253 (AKZO N.V.) * The entire document * - - -	5-7 1-3,21-22 8-20	C 12 Q 1/04 C 12 M 1/34
Y	EP-A-0 054 001 (BATTELLE MEMORIAL INSTITUTE) * The entire document * - - -	1-3,21-22	
D,A	EP-A-0 104 463 (BECTON, DICKINSON AND CO.) * Abstract; claims; figure 1 * - - -	1-22	
D,A	EP-A-0 158 497 (BECTON, DICKINSON AND CO.) * Abstract; claims; figures 1,2 * - - -	1-22	
A	EP-A-0 171 158 (UNILEVER PLC) * The entire document * - - - - -	1-22	

TECHNICAL FIELDS SEARCHED (Int. Cl.5)
C 12 Q 1/00 C 12 M 1/00 G 01 N 33/00

The present search report has been drawn up for all claims

Place of search	Date of completion of search	Examiner
The Hague	26 June 91	DOEPFER K-P.

CATEGORY OF CITED DOCUMENTS

- X: particularly relevant if taken alone
- Y: particularly relevant if combined with another document of the same catagory
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- O: non-written disclosure
- P: intermediate document
- T: theory or principle underlying the invention
- E: earlier patent document, but published on, or after the filing date
- D: document cited in the application
- L: document cited for other reasons
- &: member of the same patent family, corresponding document